



Study Protocol

Study Title: Monitoring Diversity of COVID-19 Vaccine Induced Immunity

Study Investigators:

Public Health Agency of Canada/ National HIV & Retrovirology Laboratory: Dr. T. Blake Ball¹, Dr. Catherine Card¹, Dr. Paul McLaren¹, Dr. Sandra Kiazzyk¹, Dr. Ruey-Chui Su¹, Dr. Hezhao Ji¹

External/ University of Manitoba Collaborators: Dr. Yoav Keynan², Dr. Lyle McKinnon², Dr. Derek Stein², Dr. Kelly MacDonald², Dr. Joe Bueti³

¹National HIV and Retrovirology Laboratory, National Microbiology Laboratory, Public Health Agency of Canada

²University of Manitoba, Department of Medical Microbiology and Infectious Disease

³University of Manitoba, Section of Nephrology, Max Rady College of Medicine

Background:

The COVID-19 pandemic has led to a very rapid development of novel vaccines for the SARS-COV-2 virus. The first two vaccines to show over 90% efficacy in phase III trials include novel mRNA vaccines produced by Pfizer /BioNtech¹, and Moderna. Although these vaccines have, or likely will soon been approved for use in Canada (Pfizer approved on December 9, 2020, Moderna under review), the details of the immune response to the vaccine remain unclear. In particular, questions remain regarding the duration of protection, the correlates and mechanisms of protective immunity, and the durability of immunity among those with immune-compromising comorbidities.

Vaccine efficacy and population-level immunity to infection requires both effective antibody responses (mediated by B-cells) and cell-mediated immunity (CMI; mediated through T-cells). The Pfizer vaccine, BNT162b1, a lipid nanoparticle-encapsulated mRNA vaccine targeting the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, has been shown to produce robust neutralizing antibody responses in a wide range of individuals of diverse age and ethnicity², consistent with responses observed in recovered convalescent COVID-19 patients. Less is known about the T cell-mediated CMI response. A small study (60 participants) has shown T cell responsiveness to the vaccine in those under 55³. These individuals were all healthy and had little ethnic diversity. Furthermore, the assessment of the T cell response was very basic and no information on the T cell subtypes, phenotypes or function (other than interferon (IFN)-gamma production) was assessed.

Although the presence of antibodies is commonly used as an indicator of protective immunity following vaccination or infection, it may not be the only, nor the key, correlate of immune protection. Indeed, CMI responses are necessary for mounting proper antibody responses in addition to other immune functions. CMI responses to viral infection generally involve different classes of T cells including CD8+ cytotoxic T lymphocytes (CTLs), which kill infected cells, and CD4+ T-helper (Th) cells, which vary by subset in their ability to enhance CTL and antibody responses (Th1 and TFH), modulate immunity at mucosal surfaces (Th17) and regulate these responses and limit tissue damage (Tregs)⁴. Each of these T cell subsets is critical for an effective and long-lasting immune response; however, their function varies between individuals due to intrinsic factors, including genetic background, infection history, lifestyle and comorbidities. Variability in CMI responses has been observed in COVID-19 patients and has been associated with disease severity⁵. This variability can similarly be expected to impact the ultimate efficacy of a SARS-CoV2 vaccine.

Much remains to be learned about the immune response to the new COVID-19 vaccines. This study will aim to assess the diversity and complexity of the CMI response to the COVID-19 vaccine. Importantly, we will monitor vaccine induced responses in both healthy groups, as well as those with co-morbid conditions including diabetes and kidney disease.

Objectives:

This study aims to address the following three objectives:

1. Longitudinal evaluation of the development of CMI responses in response to SARS-CoV-2 Vaccine: T cells isolated from the blood of COVID-19 vaccine recipients will be evaluated for their functionality in response to vaccine antigens. The temporal and functional properties of CMI responses will be correlated with the humoral or antibody responsiveness. CMI responses will be measured in vaccine recipients prior to vaccination to determine whether the presence or functionality of pre-existing responses to common cold coronaviruses (CCCs) or previous SARS-CoV-2 infections affect the development of CMI responses to the COVID-19 vaccine.
2. Identification of cellular and soluble factors that influence vaccine responsiveness:

While we know poor clinical outcomes in COVID-19 patients are strongly associated with markers of systemic inflammation, the influence these systemic markers will have on COVID-19 vaccine responsiveness is not clear. Using systems biology approaches, we will perform comprehensive profiling of cellular immune subsets, inflammatory signatures to identify determinants influencing the development of CMI responses to vaccine.

3. Examine variability of immune and viral genes and their relationship to vaccine induced immune responses: Human leukocyte antigen (HLA), T cell receptor (TCR) and

B cell receptor (BCR) proteins are highly genetically diverse and critical to development of protective immunity. We will perform HLA sequencing on whole blood-derived DNA samples and TCR and BCR sequencing on sorted, SARS-CoV2 vaccine antigen-specific T cells and B cells, respectively, to assess how different sequence combinations impact the CMI responses to vaccine.

Methodology

a) Study Populations

Study populations will include individuals being administered one of the approved SARS-CoV-2/ COVID-19 vaccines. Several clinics within the Health Sciences Centre and microbiology laboratory staff in Winnipeg will be accessed to include both healthy participants as well as those with underlying health conditions known to increase risk for COVID-19 disease severity.

Front line health care workers (hospital or laboratory) n=100

In partnership with Dr. Yoav Keynan who is currently running an occupational health screening program exposed health for COVID-19 care workers (HCW) at the Winnipeg Health Sciences Centre, we will enroll participants scheduled to be receiving the COVID-19 vaccine. In addition we will work with Dr. Derek Stein at the Cadham Provincial Laboratory to facilitate enrollment of laboratory workers scheduled to receive the vaccine.

We will also enroll study participants from the National Microbiology Lab, including staff members at the Canadian Science Centre for Human and Animal Health and the JC Wilt Infectious Diseases Laboratory in Winnipeg who are scheduled to receive the COVID-19 vaccine

Dialysis Patients n=100

In collaboration with Dr. Joe Bueti who heads the renal program at the Winnipeg Health Sciences Centre, we will enroll dialysis patients from the renal program scheduled to be receiving the COVID-19 vaccine.

b) Recruitment: The study protocol, recruitment, consent to participate forms, and use of data are intended to comply with ethical principles set out in the 2nd edition of the Tri-Council Policy Statement (TCPS 2) for the ethical conduct for research involving humans⁶.

Participants at least 18 years of age or older attending the various clinics will be asked by a clinic nurse/physician if they are willing to participate in a study on vaccine induced immunity. If they agree to participate, a study nurse will approach the participant using the verbal script for recruitment. Participation in the study is entirely voluntary and participants can withdraw from the study at any time. Participants receiving any Health Canada approved COVID-19 vaccine are eligible to participate.

c) Informed Consent: The study nurse will describe the nature of the study, provide a brief description of the use of the samples, and discuss issues of confidentiality, and any risks or benefits associated with the provision of the samples. All participants must provide informed consent prior to the collection of samples.

d) Questionnaire/Study Instruments

Participant Enrollment Questionnaire: Together with the research nurse, participants will be asked to fill out a basic questionnaire at the time of enrollment. This form will collect basic demographic and clinical information critical for analysis of potential drivers or confounders of observed CMI responsiveness to vaccine:

- Date of enrollment and first sample collection
- Demographics: Age, sex, height, weight, ethnicity
- Pre-existing co-morbidities
- Current Medications
- Whether participant received annual flu shot

Participant Follow Up Questionnaire : On subsequent study visits, returning participants will be asked to answer some questions on whether they experienced any side effects following vaccination or if they have experienced any COVID-19 like symptoms or have had COVID-19 test since their last study visit.

Data Extraction Form: Participants who consent will have relevant clinical data extracted from their medical records. A research coordinator will extract information from patient charts pertaining to pre-existing comorbid conditions and the clinical measurements used to monitor the severity and treatment of that condition. These will then be used to determine if they are associated with CMI responses to vaccine in multivariate analysis.

e) Sample Collection

Participants will be approached prior to receiving their first dose of COVID-19 vaccine, and will consent to sampling pre and post vaccination. The specimens to be collected include:

At each sampling timepoint, study personnel will collect:

- 4 tubes of EDTA blood: taken from a vein in the arm. This will be used to isolate immune cells for evaluation of CMI responses (Objective 1), cellular biomarkers

(Objective 2) and analysis of immune genes (Objective 3), and blood plasma for analysis of soluble biomarkers (Objective 2). Collected at all time points

- 1 NP swab in one nostril: a swab is inserted along the nasal septum to the nasopharynx and rotated for approximately 5 seconds to collect secretions then deposited into a tube containing transport medium. This will be used to evaluate cellular and soluble biomarkers (Objective 2). Note that an NP swab will ONLY be collected at time points 1, 3 and 4.
- 1 buccal swab: a swab is inserted into the mouth and brushed against the inside of the cheek then deposited into a tube containing transport medium. This will be used to evaluate soluble biomarkers (Objective 2). Collected at all time point
- 1 saliva sample: participant spits saliva into a collection tube. This will be used to evaluate soluble biomarkers (Objective 2). Collected at all time points

f) Sampling Schedule

1. **Day 1:** (Baseline sample) At the time of enrollment / prior to receiving the first dose of the COVID-19 vaccine
2. **Day 3-4:** After the initial first vaccine dose
3. Day 10-14 post first vaccine dose
- 4.
5. ~~Day 21: Prior to receiving the second vaccine dose~~ **4. Day 10-14 post second vaccine dose** : Approximately 2 weeks post second vaccine dose **2 month post second vaccine dose**
6. **4 months post second vaccine dose**
7. **6 months post second dose:** Long term sampling timepoint at 6 months
8. **1 year post second vaccine dose;** : Long term sampling timepoint at 1 year

g) Logistics and Approvals

Samples will be transported by study personnel to the JC Wilt Infectious Diseases Research Centre where researchers will process and store specimens for further analysis.

Necessary study approvals and agreements will be in place prior to commencing participant enrollment. These include impact assessments for accessing patients and health records at the Health Sciences Centre, and approvals from the Assembly of Manitoba Chiefs, Health Information Research Governance Committee (HIRGC), as we are collecting ethnicity data.

h) Testing and Analyses

The different biological readouts for our study will vary by type of test and analysis, as outlined below.

Objective 1 – CMI responses: Cells isolated from the blood samples collected will be exposed to the COVID-19 vaccine antigens or other common viruses as a comparison (e.g. CCCs, influenza). The ability of the cells to produce immune mediators in response to viral antigens will be quantified by flow cytometry. Global responsiveness to vaccine antigen stimulation will also be assessed via transcriptomics (mRNA profiling). These responses will be compared to similar responses observed in natural infected individuals with disparate disease outcome (Separate PHAC study) as an indicator of vaccine response success. CMI responses will be correlated with the humoral or antibody responsiveness tested by a number of serological assays / platforms including: two different POC rapid cassette / lateral flow antibody assays, the Roche NP and quantitative anti-RBD platforms and also by plaque reduction neutralization and /or surrogate neutralisation assays.

Objective 2 – Inflammatory Signatures: Plasma samples, swabs and saliva will be analyzed for levels of inflammatory cytokines, chemokines, biomarkers of vascular dysfunction and profiles of regulatory RNA in serum exosomes. Inflammatory biomarkers will be evaluated as determinants of CMI responsiveness to vaccination.

Objective 3 - HLA sequencing will be performed on whole blood-derived DNA samples from infected individuals. TCR and BCR sequencing will be performed on sorted, SARS-CoV-2 vaccine-specific T- and B-cells using the 10X Genomics platform to assess how different combinations impact infection outcome. Genetic sequencing data cannot and will not be used to identify individuals.

Analysis Plan:

The data collected from these various objectives will be assembled to develop models for successful CMI responses to COVID-19. Demographic and clinical variables collected will be used to stratify study groups and will be incorporated into multivariate analysis models in consultation with a biostatistician to identify confounders or true associations. In addition, should the study numbers allow, we will stratify data based on vaccine administered to identify variations of immune responses to different vaccine formulations (ie: Pfizer vs Moderna).

As we do anticipate over-representation of indigenous ethnicity among participants enrolled in the dialysis clinic, even though stratification based on ethnicity is not a primary objective of this study, should we observe any differences in vaccine responses due to ethnicity, this data may serve as preliminary data for future research to be done in consultation with indigenous health research representation.

i) Biological Sample Management

Following collection, samples will be processed at JC Wilt Infectious Diseases Research Centre and stored for subsequent analysis. Biological samples may be stored for up to ten years, and in accordance with Canadian regulations.

Ethical considerations

a) Participant confidentiality

At the time of study enrollment, participants will be given a unique study ID code to keep their identity confidential. At no time will their personal identifiers be recorded on any specimen vials or tubes which will only be labeled with participant study number and date to protect confidentiality. Only the study coordinators will know participant names and corresponding study codes. The consent forms will be kept in a separate, secured file cabinet in paper copy only. All electronic research records will be computer password protected and accessible only to research personnel. At no time will the laboratory staff who ultimately will utilize the samples have access to the name or other identifying information of the individual who provided the sample. Any information published or presented at public forums as a result of the research will not use personal identifiers, and results will be presented as a grouping of data. None of the information stored in study computers or notes will contain the participant's name, and only study numbers will be included. Study records will be retained for up to 10 years following completion of research study, after which, records will be sent for proper disposition.

Participants in the study must consent to and understand that they will not receive any of the results or data directly relating to the use of their collected samples. Participant identity will be anonymous to the researchers using their samples.

b) Potential harms, discomforts, and inconveniences to study participants

The collection of the nasopharyngeal swab involves insertion of a small swab into each nostril to the back of the throat for approximately five seconds. This may be uncomfortable but should not be painful. Blood collection involves insertion of a needle into a vein in the arm so that the blood can be drawn in to a sample tube. There may be momentary pain associated with the needle stick and occasionally people experience some bruising around the needle site. Although the needle site will be sterilized, blood collection carries a very minimal risk of infection at the site. The collection of saliva and buccal swabs are not anticipated to present any risk, discomfort or harm.

c) Potential benefits to participants

There is no direct benefit to the participant. Individual experimental results will not be communicated back with the participants

d) Expected outcomes and proposed efforts for knowledge translation

Results from this study will help to better characterize the cell mediated immune response to COVID-19 vaccination. Importantly, by monitoring vaccine response in both healthy participants and those with immune compromising underlying health conditions, we will be able to answer questions about the quality of the vaccine induced immune response in the patient groups most at risk for severe COVID-19 disease outcomes. In addition, we hope to identify strong correlates of vaccine immunity, which can be used to evaluate the success of new vaccine candidates, and survey population level immunity.

e) Potential biases

We declare no conflict of interest associated with this study.

References

1. Polack et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *NEJM* 2020
2. Mulligan, M. J. *et al.* Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 586, 589–593 (2020)
3. Sahin, U. *et al.* COVID-19 vaccine BNT162b1 elicits human antibody and T H 1 T cell responses. *Nature* 586, 594–599 (2020).
4. DuPage and Bluestone. Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. *Nat Rev Immunol* 2016; 16(3): 149.
5. Moderbahr et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* 2020; 183:996.
6. Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018). https://ethics.gc.ca/eng/policy-politique_tcps2-eptc2_2018.html (accessed on 9 December 2020).